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ON THE NATURE OF THE CANALICULAR APPARATUS OF ANIMAL CELLS.[1]

R. R. BENSLEY.

During the twelve years that have elapsed since the discovery by Golgi of the internal reticular apparatus of the nerve cell, much attention has been devoted by investigators to the consideration of similar reticular structures in cells. Our knowledge has progressed along several different lines, which have been determined more or less by the techniques employed by different investigators, and although all are not agreed as to the identity of the structures so revealed, their consideration together seems to be justified by the great similarity in configuration and location which these elements possess.

It is not necessary in this paper to review in extenso the literature on this topic, for that is well covered by the summary given by Holmgren ('02) in Merkel und Bonnet, Ergebnisse, etc., in which also the different theories of the nature of these structures are well brought out. The more recent contributions are considered in the article of von Bergen ('04) and that of Legendre ('08–'09). It will suffice here to summarize the progress that has been made along the different lines of investigation, and to consider the interpretations of these structures which have been advanced by different workers.

Golgi ('98) first described the internal reticular apparatus in the cells of Purkinje of the cerebellar cortex, where he demonstrated it by means of a modification of his well-known chrome silver impregnation method. He describes it as a closed net of fine fibers occupying the intermediate zone of the cell protoplasm and separated by a distinct interval from the nucleus on the one hand and from the surface of the cell on the other, that is to say, there was a zone of protoplasm on the periphery of the cell which was wholly free from the fibers constituting the network. Toward the nucleus the net sent out fine fibers so that the perinuclear space was not wholly devoid of fibers.

[1]From the Hull Laboratory of Anatomy, University of Chicago.

In his later studies Golgi confirmed this result for other types of nerve cells including spinal ganglion cells, spinal cord cells and cells of the cerebral cortex. In some of these cases he found the network provided with freely ending branches which terminated in a small swelling. In some cases, too, the fibers of which the network was composed had varicose swellings on them, and nodal enlargements, and, in some cells, he even found two concentric nets which differed inter se by the amplitude of the meshes and the size of the fibers. Golgi in all of his papers expressed himself with great reserve as to the nature of the network, but was confident that they had nothing to do with the neurofibrils and that they were not canals which had been filled with the silver precipitate. He was moreover certain that they were entirely intracellular and that they had no communication with extracellular structures.

In the meantime Golgi himself and his students had been extending the field of investigation to other than nervous tissues, and it had developed from these investigations that the reticular apparatus was not confined by any means to nerve cells but was present in a large variety of cells from different sources. For example, Negri ('00) demonstrated an apparatus of this sort in the cells of the pancreas and of the parotid gland of the cat, and in the thyroid epithelium of the dog. In these cells the situation of the network was quite characteristic and recalled the observations of Golgi that in young nerve cells with excentric nucleus the reticular apparatus was also excentric and located for the most part at one pole of the nucleus. In the epithelial cells, namely, it was found that the reticular apparatus was located near the nucleus, but between the latter and the free border on the lumen or surface, that is, it was distal to the blood vessels. Later, similar nets were found in the cells of the epididymus by Negri, in cartilage cells by Pensa ('01), and in striated muscle fibers by Veratti ('02).

The observations of Golgi were confirmed by a number of observers, using this method or one of the silver reduction methods of Cajal. Retzius, for example, obtained good impregnations of the apparatus in the nerve cells of the cat and rabbit, which corresponded in their salient characters with those of Golgi but

were not nearly so complete judging by the figures published. In view of this it is surprising to note that Retzius ('00) found, in some of the cells, the fibers of the network communicating by a branch with the surface of the cell, which Golgi had never observed in his more perfect preparations.

In a recent publication Golgi has adopted a new method for the demonstration of the reticulum which is based on the silver reduction method of Cajal. In view of this fact it is proper to mention here that Cajal ('07) has also studied the reticular apparatus in the nerve cell, which he accepts, contrary to Golgi, as a tubular apparatus to which he applies the name "Conduits de Golgi-Holmgren," thus accepting the interpretation of Holmgren that they are the same as the so-called juice canals described by him. He regards the appearances seen in his preparations as due to the presence of canals filled with a coagulable substance which has an affinity for colloidal silver. He also notes differences in the behavior of the apparatus in different animals, from which he concludes that the contents of the canals in different cells may have different chemical properties.

By the same methods as those employed by Cajal, Sanchez demonstrated an exceedingly interesting system in the striated muscle fibers of mammals and insects. In the former this system did not communicate with structures outside of the cell but sent free ending branches which terminated just under the sarcolemma. In the insects, however, he made the surprising observation that the intercellular network was in continuity with the tracheal system.

The second line of progress in the study of the reticular apparatus began with the discovery by Kopsch that it could be stained by prolonged immersion of the tissues in a two per cent. solution of osmic acid. In a short paper (Kopsch, '02) he described his method and contributed the results of his application of it. The results obtained corresponded very closely to those of Golgi but were obtained with greater certainty. Like Golgi he was unable to find any communications between the apparatus and the surface of the cell, although in addition to the osmic acid method he employed the resorcin-fuchsin method of Holmgren, to which reference will be made later.

The method of Kopsch has been exploited in particular by Misch ('03), and von Bergen ('04). The former found that the apparatus was not present in all cells and that in some cells it presented itself in the form of fragments, or of rows of granules. He found, moreover, in agreement with Golgi and Kopsch that the network never communicated with the surface of the cell, nor did it penetrate the nucleus. Von Bergen's studies extended to a very large category of cells ranging from wander cells to nerve cells. To show how general these structures are in animal cells a list of the elements in which von Bergen obtained positive results would have considerable interest. In addition to nerve cells he found a reticular apparatus in the following elements: prostate epithelium, pancreas cells, demilunes and mucous cells of the submaxillary gland of the cat, glandular epithelium from the trachea, chief cells of the fundus glands of the stomach, ciliated epithelium of the trachea, epithelium of the sweat glands, wander cells and many leucocytes, fixed connective tissue cells, cartilage cells, endothelium, smooth muscle, interstitial cells of the testis. The wide range of these observations taken in connection with the observations of Golgi and Cajal and their pupils indicate that the reticular apparatus is by no means a structure confined to a single cell category but is a cell organ of almost if not quite universal occurrence in the protoplasm of animal cells.

Before passing to a review of the investigations that have been made from the standpoint of the canalicular apparatus of Holmgren and others it may be of interest to refer briefly to the studies of Golgi on the development of the reticular apparatus. In the nerve cells of the fœtal calves of two or three months, he found the apparatus greatly reduced, often consisting of but a single fiber, with short branches running in various directions. In these cells the apparatus has a distinctly excentric position at one pole of the nucleus. In the new-born animal the net often extended around the nucleus, but left the perinuclear zone as well as the peripheral protoplasmic zone entirely free of such fibers. In old animals the apparatus was sometimes broken up into peculiar island-like fragments which however were connected with one another by single fibers. These observations suggest strongly that the apparatus constitutes a unit in its origin and developmental history.

The history of the intracellular canalicular apparatus, considered apart from the positive impregnations of Golgi and his followers, begins with the discovery by Holmgren ('99) of endocellular nets of juice-canals in nerve cells which he said was exhibited particularly well in preparations made from rabbit tissues. Almost at the same time Nelis ('99) described, in nerve cells fixed in sublimate or osmic acid and stained in iron-hæmatoxylin, etc., peculiar coil-like bands to which he gave the name "état spirémateux," the nature of which, however, remained to him fully obscure.

In a second publication (Holmgren, '99, 2) Holmgren described in greater detail the canalicular apparatus in the spinal ganglion cells of the rabbit, fixed in picric acid-sublimate and stained with toluidene blue and erythrosin. He found in these cells moderately fine canals of fairly uniform caliber which, anastomosing freely, formed a fairly dense network. The latter extended in general around the nucleus but often was found at one pole of the nucleus, more rarely at both poles. Here and there he found these canals communicating with pericellular canals, and at these points he was able to make out a distinct wall staining with erythrosin. He expressed the opinion that the canals were of lymphatic nature without however stating whether they were of extracellular or intracellular origin.

In 1899 Studnicka ('99) also described the canals in the protoplasm of the large ganglion cells of the trigeminus of *Petromyzon* and also in the spinal ganglion cells, in the nerve cells of the medulla oblongata and the cells of Reissner of the same animal. He explained the origin of the canals as due to the union of a row of vacuoles, and said that while many of the canals had smooth contours, yet in others might easily be seen the constituent vacuoles from which they had arisen. In a foot-note he remarked that he had not found in his objects the connection with extracellular structures described by Holmgren, although he admitted that the canals opened on the surface into the pericellular space.

In a series of papers dating from 1899 Holmgren has described the results of his investigations on this topic, covering a wide range of material including not only nerve cells, but cells from

various epithelia and from other sources. The existence of the canals has also been confirmed by a large number of observers including Kolster ('00), Fragnito ('00), Lugaro ('00), Donaggio (—), Pugnat ('01), Sjövall ('01), Smirnow ('01), von Bergen ('04) and others.

For comparison with the results of the Golgi and Kopsch techniques an enumeration of the different types of cells in which a canalicular apparatus has been found may be of interest. Holmgren demonstrated the canals in the following cells: gland cells of the pancreas and parotid, intestinal and gastric epithelium, epithelium of the epididymus, biliary duct epithelium, uterine epithelium, thyroid epithelium, liver cells, epithelium of the suprarenal gland. Retzius ('01) described similar canals in the giant cells of the bone-marrow, which, like Holmgren, he considered to be in direct connection with pericellular spaces.

It is to be noted that many of the objects studied by Holmgren coincide with those studied by Golgi and his pupils, and with those investigated by von Bergen, and that where this is the case, the canalicular structures described by Holmgren correspond closely in their location and in their configuration with those demonstrated by the other methods. Whatever conclusion we may reach with regard to the relation between the canalicular apparatus of Holmgren and the reticular apparatus in nerve cells, few who have studied the actual preparations made according to these different techniques in respect to epithelial cells and cartilage cells will deny their substantial identity. It is true that there are differences in the appearances obtained, but, in the opinion of many, these are sufficiently accounted for by the differences in the thickness of the sections studied in the different methods, and so, in the completeness of the apparatus which is brought to expression in a single preparation.

In his later papers dealing with these structures Holmgren has abandoned his original opinion that the canals are lymphatic in nature and constructed an entirely new hypothesis as to their nature. This hypothesis is based on the confirmation by him of the interesting observations of Nansen ('86) and Rhode ('91, '93, '95), that the nerve cells of certain Crustacea (Nansen, '86), and those of certain Gastropoda and Hirudinea (Rhode, *loc.*

cit.) were penetrated by a network derived from surrounding capsular cells. Holmgren found the nerve cells of *Helix pomatia* particularly suitable for the demonstration of these intracellular nets of capsular origin. He found here that the nerve cells were provided with a richer or poorer network of juice-canals, which were formed in the interior of a network of processes derived from other cells. He even found nucleated strands within the bodies of the nerve cell. In later publications (Holmgren, '01, '02, etc.)[1] he has developed this hypothesis on the basis of results obtained by the employment of a new method. He fixed his material in trichloracetic acid, or trichlorlactic acid, and stained it with a freshly prepared solution of Weigert's resorcin-fuchsin. By this method the protoplasm of the nerve cells stained faintly but that of the intracapsular cells stained dark violet, as did also the processes of the latter. By this means he was able to see processes of the darkly stained intracapsular cells which penetrated the nerve cells, branched within them, and anastomosed with one another, in order to produce an intracellular network. He applied this observation also to the nerve cells of vertebrates, and came to the conclusion that the latter were penetrated by processes of other cells which branched and anastomosed freely, to form in the interior of the nerve cells a "spongioplasma" which, however, in no wise belonged to the nerve cell, but was of extraneous origin. In the interior of these nets juice canals could arise, which communicated directly with similar spaces in the interior of the matrix-cells of this net. To this net of extraneous origin Holmgren gave the name "trophospongium." He regarded therefore the trophospongia not as fixed structures, but as undergoing a constant change, which depended upon the physico-chemical processes in the cell, and thought that, while at one moment the network of cell processes might sacrifice itself by liquefaction to the needs of the nerve cell, it might later be regenerated, by new growth of the process from without. He thus abandoned completely his former view that the canals represented circulatory or lymphatic structures, or a drainage system, in favor

[1]For complete references see Holmgren, E., "Neue Beiträge zur Morphologie der Zelle," in "Merkel-Bonnet Ergebnisse," Vol. 11, p. 275.

of the view that they represented the transitory phases of the reciprocal nutritive inter-relations of the capsular and nerve cells.

The object of the foregoing brief and incomplete résumé of the literature, has been to show that from three different lines of investigation we have evidence of the wide occurrence in animal cells, ranging in diversity from leucocytes to nerve cells and muscle cells, of a reticular apparatus, which exhibits itself in the form of a network of canals with colorless contents, or of a stained network according to the technique employed. The uniformity with which this apparatus has been discovered in those types of cells in which it has been sought justifies the expectation that similar methods will reveal similar structures in cells which have thus far not been investigated with this point in view. We are thus dealing with a cell organ of almost if not quite universal distribution in animal cells.

The question now arises—What is the significance of this structure?

The trophospongium theory of Holmgren, as far as I am aware, has found no support. Even if it were admitted for the nerve cells there are many categories of cells in which a reticular apparatus, or a canalicular apparatus is to be found, to which the theory is wholly inapplicable. For example, it is difficult to conceive how the reticular canalicular apparatus of the cartilage cells and of leucocytes, could be derived from the liquefaction of penetrating processes of other cells. Holmgren, it is true, has made an attempt to adapt his hypothesis to the canalicu ar apparatus of epithelial cells, and has described in the pancreas of the salamander the continuity of the intracellular network with intracellular strands which go to the periglandular connective tissue cells or to the centro-acinous cells. However, all of Holmgren's figures of preparations made by the trichloracetic acid, resorcin-fuchsin method are explainable on the basis of the canals having a precipitable content which when precipitated by the fixative, has an elective affinity for the dye. It is not by any means certain that the figures which Holmgren has given us of intracellular nets stained by fuchsin, in continuity with processes of capsular cells, do not really represent two different structures brought into apparent relation with one another by a common

affinity for the dye. It is certain, moreover, that the networks apparently composed of solid fibers demonstrated in the pancreas epithelium by the resorcin-fuchsin method, are not solid, for, in preparations made by this method, every cell in the section will show such a deeply stained network while sections of the same pancreas fixed in Kopsch's formol-bichromate solution and stained with iron-hæmatoxylin, will show in every cell a system of canals with unstained contents.

Accordingly, we must either reject Holmgren's hypothesis or assume that there are two sorts of these nets, those of cartilage cells and epithelial cells and leucocytes being different from those of nerve cells.

The statement of Legendre ('08–'09) that these structures are either wholly absent or are the result of pathological changes is not to be seriously considered, in view of the fact that this author in attempting to explain the positive observations in this regard of so many experienced investigators, is compelled to resort to the wholly unwarranted assumption that their results have been due to the selection of unhealthy animals, or to the fixation of tissues after several days of inanition, or several hours after death.

Many observers, including Retzius ('00), are inclined to believe that they represent an intracellular system of nutritive or drainage canals having direct relations with the lymphatic system. The extracellular communications are, however, denied by Golgi and his pupils, who have never seen them in their preparations made by the chrome-silver impregnation methods, nor by the silver reduction method. They are equally denied by von Bergen, who, however, admits the existence of canals in no wise connected with these, which he regards as artefacts, which do open on the surface of the cell.

Von Bergen ('04) who studied these structures by all three methods but, in particular, by the osmic acid method of Kopsch, agrees with Golgi that the structures are for the most part networks of fibers composed of a substance which reduces osmic acid, but explains the discontinuous elements found by him in many cells studied, by the assumption that they represent different stages in the formation or destruction of the apparatus

which thus would have a variable structure from moment to moment in the cell. He claims that the reticular apparatus arises by the appearance in the cell protoplasm of granules or droplets which arrange themselves in net-like or tortuous rows which fuse to form more continuous fibers, and further, that the network so formed can undergo vital changes, by virtue of which it loses its stainability and becomes dissolved, the canals so formed finally disappearing by absorption of their contents.

In view of the almost universal occurrence of these structures in all of the tissue cells of mammals, and in many of those of lower vertebrates and invertebrates, it seemed probable that they would not be wholly absent from the cells of the other great division of living organisms, namely from the cells of plants. Accordingly, I have studied with this end in view the structure of certain plant cells, using for this purpose in addition to the conventional methods of plant histology, those methods which in my experience were best for demonstration of the canalicular system in animal cells. It seemed probable, in view of the conditions found in the animal cell, that, if a homologue of the canal network of the animal cell were to be found in plant cells, it would be studied with greatest ease in those plant cells in which the vacuolar system had not yet reached its full development, namely in meristem tissues, sporogenous tissues and their products, cambium and embryonic tissues. The three last, however, did not lend themselves readily to this investigation because of the difficulty introduced by the slow penetration of the fixing agents, so that I have been obliged for the present to content myself with the results obtained in the root-tips of *Allium*, *Lilium* and *Iris*, and in the tapetum of the lily. Whether the consistent results obtained from the study of these cells are generally applicable or not to plant cells, future investigation will show. In the meantime, because of the fact that the results are at variance with the accepted views of the structure of the cells in question, because they furnish a new interpretation of the history of the vacuole of these cells and in particular because they seem to throw an interesting light on the question of the nature of the canalicular apparatus of animal cells, it seems wise to put these preliminary observations on record.

The descriptions which follow have been drawn largely from the study of preparations of the root-tip of the onion, but the observations made on the roots of the other genera mentioned are in full accord with them.

In his study of the vacuolar system of the cells of plants, Went ('88) describes the young cells of the onion root-tip as follows: "In the youngest cells of roots two or three millimeters in diameter I saw a great number of very small vacuoles; the largest had a diameter of four mikra, the smallest of one mikron." For these small vacuoles he claims reproduction by division, in the sense of the tonoplast theory of DeVries ('85). He also derives the vacuole of the older cell from these multiple vacuoles, by a process of coalescence.

In preparations made after fixation in Flemming's strong fluid, Hermann's fluid, Zenker's fluid, Carnoy's fluid, etc., and stained in iron hæmatoxylin, or in the three-color process of Flemming, results exactly corresponding to these were obtained, that is to say, the young cells contained a multitude of small vacuoles which by their coalescence seemed to form the large central vacuole of the older cell.

On the other hand, preparations made by methods which I had found to be most effective for the demonstration of the canalicular apparatus in animal cells, gave results which were wholly different. In these there was no trace in the youngest cells of the root tip of the multiple vacuoles described by Went and others, but instead, each cell possessed an intricate network of canals the component elements of which in the youngest cells were often of extreme fineness. These canals were best seen in the dermatogen cells on the surface of the root but were recognizable as such, though less well preserved, in the cells of the plerome. Fig. 1 shows a cell in which this system is composed of extremely fine canals. In this figure it will be seen that the canalicular system tends in these cells as in the animal cells to

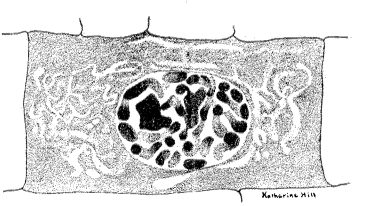

FIG. 1. Cell of outer layer of the root tip of onion, showing fine canals in the cytoplasm. × 800.

leave a peripheral zone of cytoplasm wholly free from the canals which constitute it. In Fig. 2 are shown four of the large wedge-shaped cells from the region of most active division of the dermatogen, in which the type of the canalicular system is well brought out. Here it will be seen that in these plant cells, as in the animal cells, the network tends to be concentrated on one side of the nucleus, and, as in the epithelial cells, this point of concentration is not one of the division poles of the nucleus but

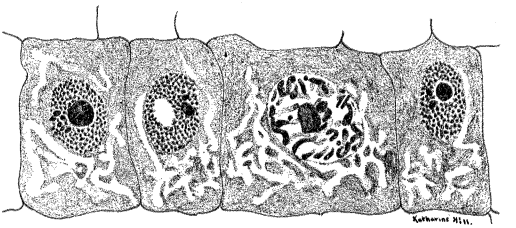

FIG. 2. Four cells of outer layer of root tip of onion, showing more advanced condition of the cytoplasmic canals. × 800.

corresponds to one side of the equator of the future spindle. The system constitutes a closed system of canals, lying in very close relation to the nucleus, never, however, invading, in dividing cells, the spindle territory. From this network run out branches which end freely often near the cell wall in a small expansion. Many of the canals in some preparations, and this is particularly true of the smallest canals, such as those shown in Fig. 1, show moniliform enlargements, as if they were on the point of breaking up into a row of vacuoles, or possibly, as if they had just been formed by the coalescence of a row of vacuoles. Again frequently, the canals show a spiral or tortuous course, as if they were fixed while in a condition of internal tension, which resembles very closely the spiral or tortuous condition found in many nerve cells (état spirémateux of Nelis).

Tracing this system in the older and older cells of the root tip it is found that as the cell retreats from the growing point, the canals become progressively larger and larger. In the intermediate stages of this process the condition depicted in Fig. 3 is obtained. Here there is still a continuous system of canals but they are fewer in number and broader than in the younger

cells. Ultimately by a continuation of this process we have the familiar picture of the plant cell with a large central vacuole across which run strands of protoplasm which are the last attenuated remains of the protoplasmic partitions between the canals.

A similar mechanism is revealed by the same technique in the tapetal cells of *Lilium candidum*. In preparations made after fixation in Flemming's fluid, the protoplasmic tip of the cell presents a foam-structure owing to the presence in it of a

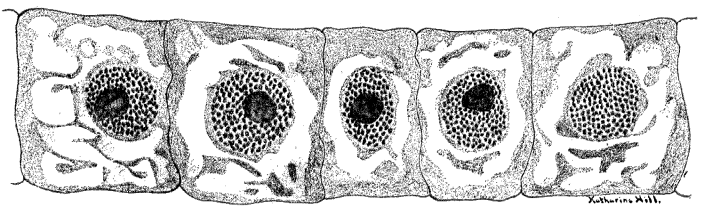

FIG. 3. Five cells of second row of root tip of onion, showing the expanded canals, beginning to form the large central vacuole.

large number of fine vacuoles. In preparations, however, which have been made by the technique referred to above, it is seen that instead of a multitude of minute vacuoles or alveolæ, there is a system of exceedingly fine canals forming a network which opens at intervals into the large vacuole which occupies the base of the cell.

Apart from the elements which constitute the tubes or vacuoles according to the method of preparation, the cytoplasm of these cells shows no indication whatever of an alveolar structure under the microscope. It is composed of an optically homogeneous ground substance in which are imbedded the mitochondria and other granular elements, for example plastids, which may be present.

The two different techniques, therefore, give us two entirely different conceptions of the history of the vacuole. According to the first the vacuole arises from the coalescence of preëxisting innumerable small vacuoles. According to the new methods, the vacuole in these cells constitutes a unit element from the very beginning, being represented in the younger cells by a single system of anastomosing canals.

The decision as to which of these views expresses the condition in the living cell must of necessity rest on the examination of living cells. Before taking up this question, however, we may discuss the significance of these observations in the interpretation of the canalicular apparatus of the animal cell for this interpretation does not of necessity imply the assumption that the canalicular structure so demonstrated in the plant cell has a real preëxistence in that form in the living cell. We may, on the contrary, treat the technique as an experimental method and discuss the results comparatively on this basis.

For the demonstration of the vacuolar system of plant cells as a network of canals I have found the following fixing fluids best adapted:

1. FORMALINE, BICHROMATE, SUBLIMATE.

Neutral formaline (freshly distilled)	10	c.c.
Water	90	c.c.
Potassium bichromate	2.5	gr.
Mercuric chloride	5	gr.

2. KOPSCH'S FLUID.

Potassium bichromate 2.5 per cent. in water	75	c.c.
Neutral formaline	25	c.c.

With these fluids, as indicated above, the cells of the root tip show a network of canals, whereas the same tissues fixed in Flemming's solution show, instead of canals, multiple small vacuoles. The same statement holds good for animal cells similarly treated. For example, the epithelial cells of the intestinal glands fixed in the formaline-bichromate-sublimate mixture, or in Kopsch's fluid, show a beautiful canalicular system, while the same cells fixed in Flemming's fluid show at the site of the canals merely a large number of exceedingly fine vacuoles. Thus whether we accept the multiply vacuolated condition, or the canalicular condition, as the preëxisting one in the living cell, the analogy between these structures in the animal and vegetable cell holds.

On the basis of the similarity in constitution of the canalicular apparatus of the plant cell to that of the animal cell, and of the similarity in behavior of this system when treated by the same methods and an account of the part these canals in the plant cell

take in the history of the vacuole of the latter, I think we are justified in stating for the present, to be sure, only as a working hypothesis, that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system of the plant cell.

We may now return to the consideration of the question whether the canalicular system represents the true structure of the vacuolar mechanism of young plant cells or not. This question can, of course, be answered only by observations of the living cells themselves, and the investigation of these is beset by extraordinary difficulties in the case of the plant cell due in particular to the impossibility of finding a solution in which to examine the cells, which is not itself injurious. In the effort to find a suitable fluid for this purpose I tried solutions of potassium nitrate, of sodium chloride, and of cane sugar of different concentrations, but found in all that the surface layers of cells showed rapid changes of the structure of the protoplasm which made it difficult to study the presumably uninjured deeper layers of the sections. I was finally obliged to resort to the expedient of using as a mounting medium the freshly expressed juice of similar tissues, although even in this the cells of the surface layers of free-hand sections underwent more slowly the same change, In these surface layers so mounted the cells showed the multiple vacuolar condition described by Went. In the deeper layers, on the contrary, in sections of the onion root tip, one could see, with difficulty to be sure, but still unmistakably the canal system represented in Figs. 1, 2 and 3. As these cells are watched, however, the canals are seen to break up slowly into rounded vacuoles thus bringing about the condition generally recognized in these cells. I regard, therefore, the canalicular system as the true condition intra vitam of the vacuolar apparatus in these cells of the root tip, and believe that the multiply vacuolated condition is of secondary origin due in most cases to injury of the cell.

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